

## New Species of *Coelastrella* and *Scenedesmus* (Chlorophyceae, Chlorophyta)

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Two green algae were collected from tree bark and coastal water. One isolated from tree bark is solitary and unicellular, and has network ribs on the cell wall. This alga is morphologically similar to *Coelastrella vacuolata* (Shihira & Krauss) Hegewald & Hanagata, but differs from it in the shape of cells and visible ornamentation on the cell wall in light microscopy. The other isolated from coastal water is in colony, and has a meridional ribs on the cell wall. The gross morphology of the second alga resembles that of *Coelastrella multistriata* (Trenkwalder) var. *corcontica* Kalina & Punčochářová, but differs from it in habitat. The phylogenetic trees constructed with sequence data of 18S rRNA gene showed that the second alga is distant from *Co. multistriata* var. *corcontica*. This alga is placed in the subgenus *Acutodesmus* of the genus *Scenedesmus*, and forms monophyletic relationship together with *Scenedesmus rubescens* (Dangeard) Kessler & al. and *S. dissociatus* (Verses & Trainor) Hegewald & Hanagata. Therefore, these two algae are described as new species, *Coelastrella saipanensis* Hanagata and *Scenedesmus littoralis* Hanagata, respectively.

**Key words:** Bark inhabiting, *Coelastrella saipanensis*, new species, *Scenedesmus littoralis*

### Introduction

The genus *Scenedesmus* is common fresh-water planktonic algae and characterized by the formation of coenobia of 2-, 4-, 8-, 16-, or rarely 32-cells arranged in one or two rows. The occurrence, arrangement and length of the spines on the surface of the cells have been used as taxonomical criteria for the classification of *Scenedesmus* for more than one hundred years. However, recently, the cell wall ultrastructure under electron microscopy is used as the most important criteria at species rank of *Scenedesmus* (Hegewald et al. 1990, Hegewald and Schnef 1991).

Hanagata et al. (1996) found single-celled

*Scenedesmus komarekii* on bark. The gross morphology of the alga is close to that of *Chlorella*, but the cell wall ornamentation revealed by electron microscopy showed that the alga is a member of *Scenedesmus*. Kalina and Punčochářová (1987) re-examined several species of *Chlorella* using electron microscope, and excluded *Ch. fusca* var. *vacuolata* and *Ch. fusca* var. *rubescens* from *Chlorella* on the basis of the cell wall structure. They assigned these two algae to *Graesiella vacuolata* and *Halochlorella rubescens*, respectively, and placed them in the subfamily Scotielloecystoideae, the family Chlorellaceae. However, Kessler et al. (1997) later transferred these algae into

*Scenedesmus* based on the phylogenetic study. Hanagata (1998) also transferred several single-celled algae known as the members of the subfamily Scotielloecystoideae into *Scenedesmus* on the basis of the sequence analyses of 18S rRNA gene. Recently, Hegewald and Hanagata (2000) reclassified the family Scenedesmaceae on the basis of morphology and phylogenetic data, and transferred these single-celled *Scenedesmus* into the genus *Coelastrella*.

Many specimens of coccoid green algae have been collected from tree bark, soil, freshwater and coastal water in this study. They were cultured and examined with light and electron microscopy. Of these, the cell walls of two algae isolated from tree bark and coastal water, respectively, have particular ornamentation patterns which are the characteristic features of the subfamily Scotielloecystoideae. In this paper, these two algae are described as new taxa *Coelastrella saipanensis* Hanagata and *Scenedesmus littoralis* Hanagata, respectively, and their systematic positions are discussed on the basis of the results obtained by light and electron microscopic observations as well as phylogenetic study.

### Materials and Methods

*Coelastrella saipanensis* was collected from the bark of *Cocos nucifera*, on 8 October, 1993, in Saipan, North Mariana Islands. *Scenedesmus littoralis* was isolated from seawater collected in the coastal area faced to the Tokyo Bay of Chiba Prefecture, Japan. The algae examined in this study were cultured in Bold's basal medium (1949). The methods of unialgal culture and scanning electron microscopy were described in a previous paper (Hanagata et al. 1996).

All information on extraction procedure of genomic DNA was provided previously (Hanagata 1998). To obtain almost complete 18S rDNA, polymerase chain reaction (PCR) protocols (Saiki et al. 1988) were used.

Primers for the amplification and PCR conditions were the same as those shown in Nakayama et al. (1996). Double strand PCR product was directly sequenced using a DNA autosequencer SQ-5500 (Hitachi Co., Ltd., Japan) according to manufacture's instructions.

The sequences determined in this study were manually aligned with other previously known 18S rDNA sequences. No ambiguities in alignment occurred. The sequences included in this study are as follows (with accession numbers): *Chlamydomonas reinhardtii* Dangeard (M32703), *Coelastrella multistriata* (Trenkwalder) Kalina & Punčochářová var. *multistriata* (AB012846), *C. multistriata* var. *corcontica* Kalina & Punčochářová (AB037082), *C. oocystiformis* (Lund) Hegewald & Hanagata (AB012848 as *Scotiellopsis oocystiformis*), *C. terrestris* (Reisigl) Hegewald & Hanagata (AB012847 as *Scotiellopsis terrestris*), *C. vacuolata* (Shihira & Krauss) Hegewald & Hanagata (X56104 as *Scenedesmus vacuolatus*), *Dunaliella salina* (Dunal) Teodoresco (M84320), *Neodesmus pupukensis* (Kalina et Punčochářová) Hegewald & Hanagata (X91267 as *Scenedesmus pupukensis*), *Scenedesmus acuminatus* (Lagerheim) Chodat (AB037088), *S. acutus* Meyen (X56103 as *S. obliquus*), *S. bacillaris* Gutwinski (X91266 as *S. producto-capitatus*), *S. dissociatus* (Verses & Trainor) Hegewald & Hanagata (AB037084 as *Dactylococcus dissociatus*), *S. distendus* (Holtmann) Hegewald & Hanagata (AB037093 as *S. pectinatus* var. *distendus*), *S. hindakii* Hegewald & Hanagata (AB037098 as *Schroederiella apiculata*), *S. obtusus* Meyen (AB037091), *S. reginae* (Holtmann) Hegewald & Hanagata (AB037096 as *S. wisconsinensis* var. *reginae*) and *S. rubescens* (Dangeard) Kessler & al. (X74002 as *S. rubescens*).

Sequences were analyzed with the maximum parsimony (MP) and distance matrix

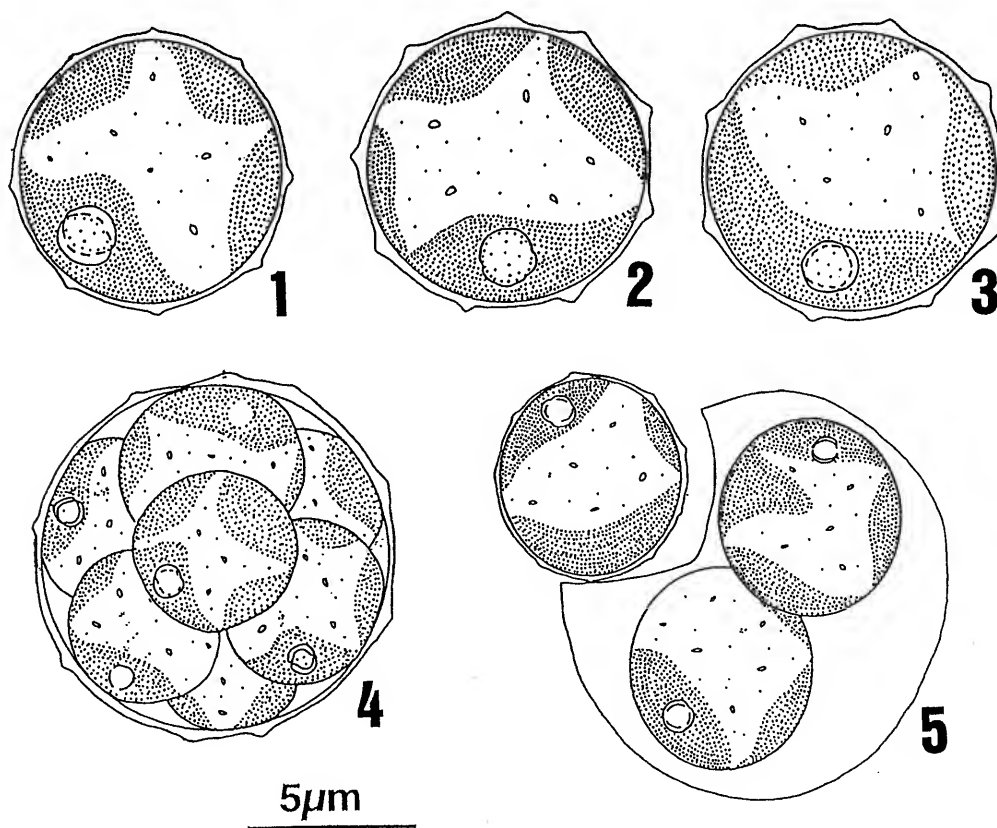
methods. The maximum parsimony analysis was implemented with the PAUP computer program (version 3. 1. 1; Swofford 1993) using a heuristic search under the unweighted scheme. A random addition of sequence with 10 replicates and a branch-swapping algorithm (TBR, or tree bisection-reconnection) was used. The same sequence alignment data were converted to a distance matrix by means of the two-parameter method (Kimura 1980) using CLUSTAL W (Thompson et al. 1994). The distance matrix was converted to a phylogenetic tree using the neighbor-joining (NJ) algorithm (Saitou and Nei 1987) of CLUSTAL W. Support for internal branches in both the trees constructed with the maximum parsimony and

neighbor-joining methods were estimated using the bootstrap method (Felsenstein 1985).

### Descriptions

***Coelastrella saipanensis*** Hanagata, sp. nov. [Figs. 1–5, 9, 10]

Alga unicellularis et solitaria. Cellulae sphaericae, 4–14  $\mu\text{m}$  diam. Paries cellulae in microscopio optico ornamentum reticulatio-nis supra superficiei habens. Chloroplastum parietale, tabuloeforme, cum uno pyrenoide. Pyrenoides sphaerica vel ovata, et granis amyli circumcincta. Cellula interdum grana oleosa et vacuolas habens. Reproductio asexualis per 2–16 autosporas magnitudinis aequalis effecta. Autosporae sphaericae, 3–7



Figs. 1–5. *Coelastrella saipanensis* Hanagata, sp. nov. 1–3. Vegetative cells. 4. Autospores in sporangium. 5. Autospores discharged from parent cell.

$\mu\text{m}$ , per aperturam parietis sporangii liberatae. Substantia carotenoidis secunda praesens.

Holotype: Figure 1.

Type locality: Saipan, North Mariana Islands.

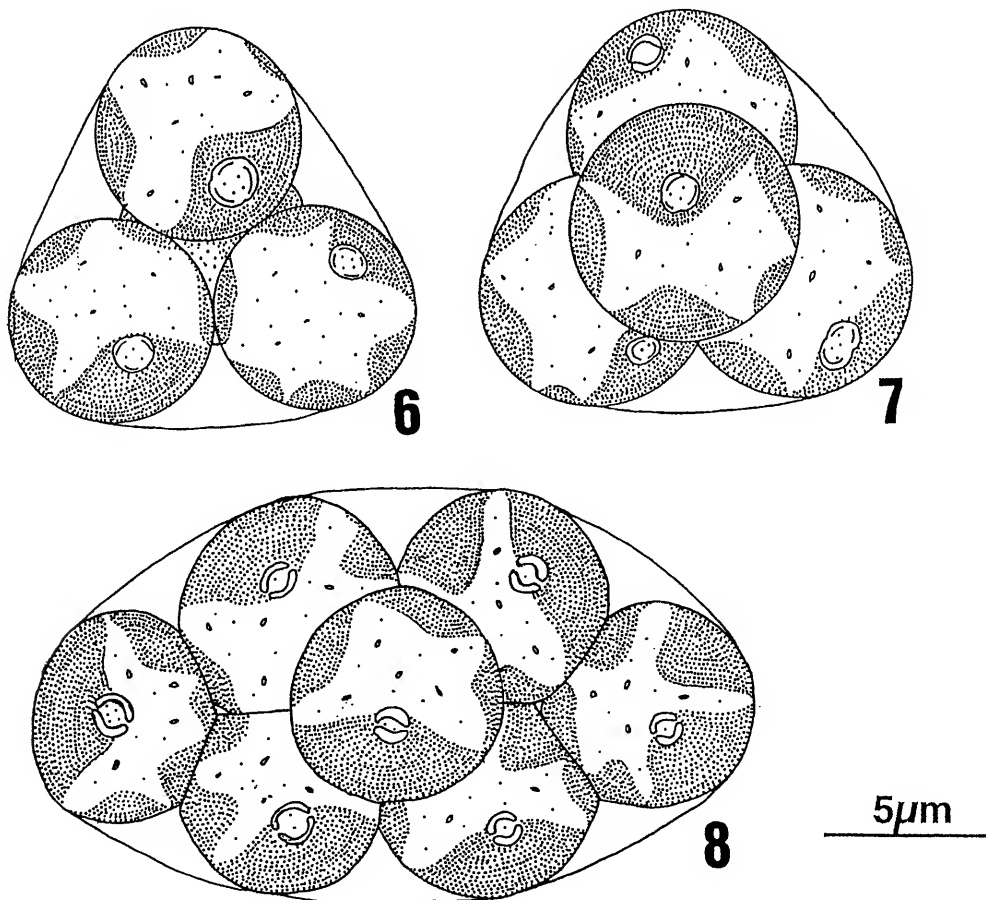
Habitat: Specimens examined were isolated from the samples attached on the bark of *Cocos nucifera*.

Type culture: Saipan, North Mariana Islands, 8 October, 1993. Strain D9-1 deposited in the Culture Collection at Research Center for Advanced Science and Technology (RCAST), The University of Tokyo.

***Scenedesmus littoralis* Hanagata, sp. nov.**

[Figs. 6–8, 11, 12]

Alga colonialis 2–4–8 cellulis formans. Cellulae sphaericae, 3.5–12  $\mu\text{m}$  diam, vel ovatae, 3–8  $\times$  3.5–10  $\mu\text{m}$ . Paries cellulae ornamentum meridianum supra superficiei habens. Chloroplastum parietale, cum uno pyrenoide. Pyrenoides sphaerica vel ovata, et granis amyli circumcincta. Cellula saepe grana oleosa habens. Cellulae maturae vacuolas numerosas habentes. Reproductio asexualis per 2–16 autosporas. Autosporae ovatae, semisphaericae, per aperturam parietis sporangii liberatae. Substantia carotenoidis secunda praesens.



Figs. 6–8. *Scenedesmus littoralis* Hanagata, sp. nov. 6, 7. Four-celled colonies. 8. Eight-celled colony.

Holotype: Figure 6

Type locality: Yawata-kaigan, Ichihara, Chiba Prefecture, Japan.

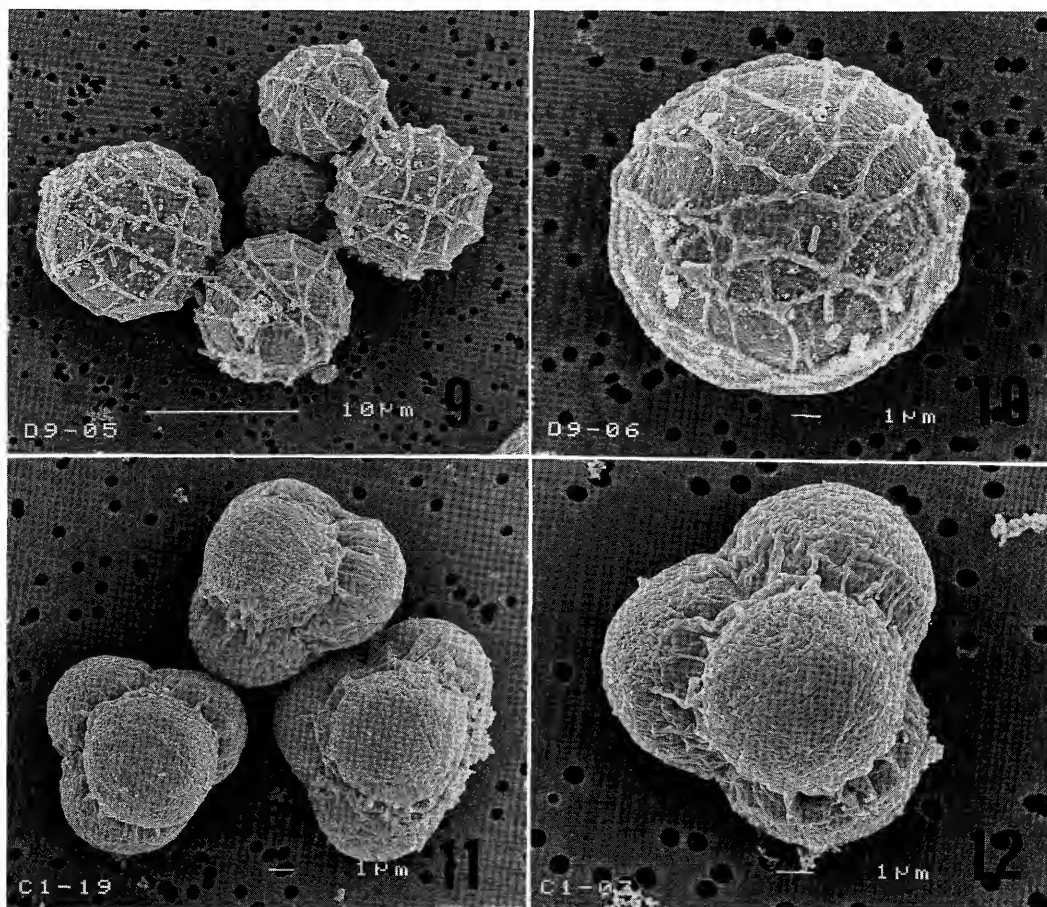
Habitat: Specimen examined was isolated from the coastal water.

Type culture: Yawata-kaigan, Ichihara, Chiba Prefecture, 6 April 1997. Strain CI deposited in the Culture Collection at Research Center for Advanced Science & Technology (RCAST), The University of Tokyo.

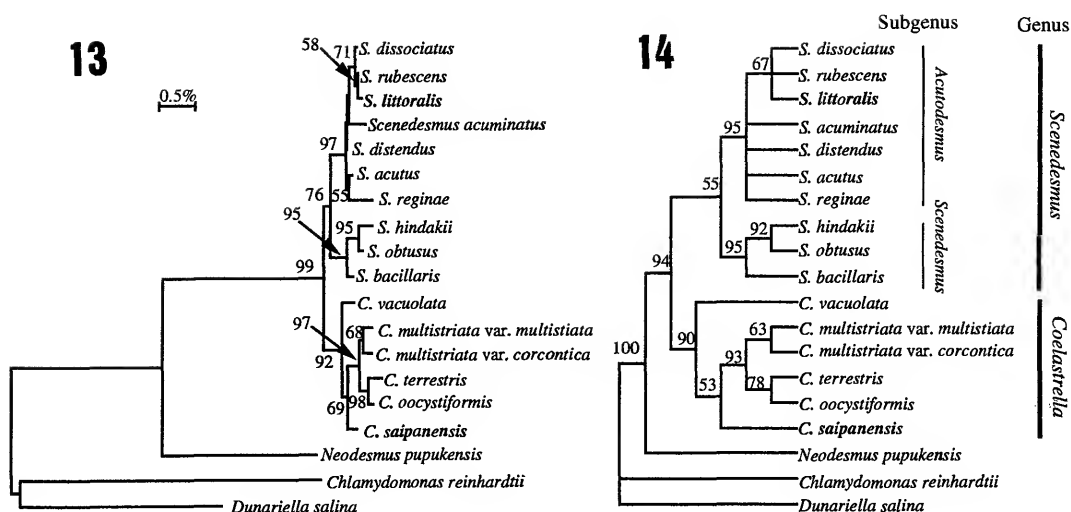
*Scenedesmus littoralis* is able to grow in the salinity up to 2 % NaCl, although the maximum growth rate is obtained in the culture medium without added NaCl.

### Phylogenetic positions

The phylogenetic tree obtained from NJ analysis is shown in Fig. 13. MP analysis resulted in fifteen equally parsimonious resolutions (length = 286 steps, consistency index = 0.914). A strict consensus tree is presented in Fig. 14. *Coelastrella saipanensis* (AB055800) is placed in a monophyletic lineage of the genus *Coelastrella*. Two varieties of *Co. multistriata* form a monophyletic relationship to a group consisting of *Co. terrestris* and *Co. oocystiformis*. *Coelastrella saipanensis* forms a sister relationship to these combined. *Coelastrella vacuolata* first



Figs. 9, 10. Scanning electron micrographs of *Coelastrella saipanensis* Hanagata, sp. nov. Figs. 11, 12. Scanning electron micrographs of *Scenedesmus littoralis* Hanagata, sp. nov.



Figs. 13, 14. Phylogenetic trees inferred from 18S rDNA sequences. The numbers on the branches indicate bootstrap values (1000 replicates). 13. A distance tree constructed with the neighbor-joining method. 14. A strict consensus tree generated by heuristic search.

diverges in this lineage. *Scenedesmus littoralis* (AB055801) is placed in the lineage of the subgenus *Acutodesmus* in the genus *Scenedesmus*. *Scenedesmus littoralis* forms a monophyletic group together with *S. rubescens* and *S. dissociatus*.

### Discussion

The size of cells, the shape of chloroplast and the cell wall ornamentation of *Coelastrella saipanensis* resemble those of *Co. vacuolata*. The new species, however, differs from *Co. vacuolata* in the shape of cells and ornamentation pattern on the surface of the cells. In *Co. vacuolata*, cells are often broadly ellipsoidal in shape and the cell wall is smooth when viewed by light microscopy (Kalina and Punčochářová 1987). On the other hand, in *Co. saipanensis* the cells are always spheroidal in shape and the network of ribs on their surface can be observed even by light microscopy. The cell wall ornamentation of *Co. saipanensis* is also similar to that of *Scotiellopsis reticulata* as observed by Punčochářová and Kalina

(1981), but differs from it in the absence of polar thickenings.

The characteristic features of *Scenedesmus littoralis* are the presence of meridional ribs on the cell wall and the formation of coenobial colony. The ornamentation pattern of cell wall in this species is similar to that of *Co. oocystiformis*, *Co. terrestris* and *Co. multistriata* var. *multistriata*, but differs from them in the formation of colony. *Coelastrella compacta* Skuja (1959) also has meridional ribs and forms a similar colony, but the cells of this species are larger than *S. littoralis*. In addition, one of the cell poles of *Co. compacta* is provided with a papilla, while *S. littoralis* does not have such a structure. The morphological features including the shape and the size of cells, and the shape of chloroplast of *S. littoralis* basically resembles those of *Co. multistriata* var. *corcontica* which has meridional ribs on the cell wall and forms colonies. The difference between *S. littoralis* and *Co. multistriata* var. *corcontica* is their habitats, the former inhabits coastal water while the latter inhabits peat

pools in the subalpine region (Kalina and Punčochářová 1987). The phylogenetic trees in this study show that *S. littoralis* is distantly related to *Co. multistriata* var. *corcontica*, although these two species are morphologically similar to one another. *Scenedesmus littoralis* is phylogenetically close to *S. rubescens* and *S. dissociatus*. *Scenedesmus rubescens* is also halotolerant (Kessler 1982), but the species differs from *S. littoralis* in the following morphological features; the shape and size of cells, the cell wall ornamentation, and the shape of chloroplast. The solitary cells of *S. rubescens* are sometimes connected polarly by appendages to short chains (Kalina and Punčochářová 1987). In addition, this alga has polar thickenings and often develops appendages, composed of single microfibrils. It was first described as *Halochlorella rubescens* by Dangeard (1965), and later transferred into *Chlorella* by Kessler et al. (1968). Kessler et al. (1997), however, re-transferred this species into *Scenedesmus* on the basis of phylogenetic study as described above. *Scenedesmus littoralis* is also distinguishable from *S. dissociatus* by gross morphology. *Scenedesmus dissociatus* was isolated from farmland soil and originally described as *Dactylococcus dissociatus* (Verses and Trainor 1966). This species is spindle-shaped with bristles often connecting the cells to chains. The halotolerance of the species has not been verified. The phylogenetic analysis revealed that *S. littoralis* is a member of the subgenus *Acutodesmus*. One of the morphological features of *Acutodesmus* is the shape of cells which are spindle-like with acute cell poles (Hegewald and Hanagata 2000). While the cells of *S. littoralis* are spheroidal or ovoidal, and do not share the morphological feature with those of *Acutodesmus*. *Scenedesmus littoralis* seems to be morphologically an exceptional taxon in the subgenus *Acutodesmus*.

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#### 花方信孝：Coelastrella 属及び Scenedesmus 属（緑藻綱）の2新種

北マリアナ諸島サイパン島の樹木皮および東京湾内房地区の沿岸海水より単離した鞭毛をもたない2種の緑藻について分類学的な研究を行った。樹皮から単離した藻は単細胞で、細胞は球形であり、細胞壁表面には子午線状の隆起を有する。Coelastrella vacuolata に類似するが、細胞壁表面の隆起が光学顕微鏡でも観察できる点で異なる。この藻は、18S rDNA 領域の塩基配列の解析では Coelastrella 属に位置するが、近縁の既知種がないことから新種と認め、Coelastrella saipanensis

Hanagata とした。沿岸海水より単離した藻は2–4–8細胞のコロニーを形成し、細胞表面には子午線状の隆起を有する。形態は Co. multistriata var. corcontica に類似するが、食塩を含む培地でも増殖できる点で異なる。この藻は分子系統上は Scenedesmus 属 Acutodesmus 亜属に位置するが、この亜属に類似の形態的特徴をもつ既知種がないので新種と認め、Scenedesmus littoralis Hanagata とした。

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